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Formulation And Evaluation Of Phytosoamal Gel Of *Neem*

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ABSTRACT

Phytosomes of neem leave extract has been successfully formulated in this procedure firstly *Azadirachta indica* leaves were morphological Characterized. Neem leave powder was subjected for various physiochemical analysis then extracted out methanolic fraction of neem leaves, yield of the extract which was 6.34 %. Phytochemical screening found that carbohydrate, alkaloids and flavonoids were present. then extracted out methanolic fraction of neem leaves, yield of the extract which was 6.34 %. Phytochemical screening found that carbohydrate, alkaloids and flavonoids were present. Total phenolic compound (TPC) was 0.756mg/100mg of gallic acid equivalent of dry extract sample, Total alkaloid content was atropine equivalent 0.632mg/100mg. Five different type of phytosome formulations were formed using different ratio of Phosphatidylcholine and Cholesterol (4:1), (2:1), (4:3), (1:1) and (1:1.25). These formulations were characterized under various parameters like yield, drug content, particle size and Encapsulation Efficiency. For all formulation yield was 88.32 % to 98.91%, drug content was 90.21% to 97.52 %, Mean Particle Size(nm) about 700 nm and Encapsulation Efficiency was 78.67% to 95.34%. Drug: Excipient Compatibility confirmed by FT-IR studies. All five phytosome formulations were incorporated with gel and evaluated under the various parameter, pH of all formulations was observed between 6.8 to 7.3 and Spreadability between 5.6 to 7.9 cm. % drug content between 98.9 % to 101%, and viscosity between 98 to 115 centi poice (cp) and % permeation between 83.2 % to 92.7 %. But on the basis of drug release kinetics F-3 formulation was very good because its % drug release was 97.913% followed Higuchi Kinetic Model. With the 95.56% drug release F-2 formulation was good both follow First order Kinetics.

Keyword: *Azadirachta indica,* physiochemical, phytosome, gel, neem,

INTRODUCTION

Novel herbal drug carriers cure particular disease by targeting exactly the affected zone inside a patient's body and transporting the drug to that area[1]. Novel drug delivery system is advantageous in delivering the herbal drug at predetermined rate and delivery of drug at the site of action which minimizes the toxic effects with the increase in bioavailability of the drugs[2]. Phytosomes are novel complexes which are prepared by reacting from 3-2 moles but preferably with one mole of a natural or synthetic phospholipid such as phosphatidylcholine, phosphatidyl ethanolamine or phosphatidyl serine with one mole of component for example flavolignanans[3], either alone or in the natural mixture in aprotic solvent such as-dioxane or acetone from which complex can be isolated by precipitation with non-solvent such as aliphatic hydrocarbons or lyophilization or by spary drying. In the complex formation of phytosomes the ratio between these two moieties is in the range from 0.5-2.0 moles[4,5]. A gel is a two-component, cross linked three-dimensional network consisting of structural materials interspersed by an adequate but proportionally large amount of liquid to form an infinite rigid network structure which immobilizes liquid continuous phase within[6,7].

Neem is a very beneficial plant that is used to cure many diseases[8]. Every part of the neem tree like neem seed, leaves, bark, roots, twigs can be used for medicinal purposes[9]. For better and improved bioavailability, natural phytoconstituents must have a good balance between hydrophilicity and hydrophobicity [10]. This is achieved through phytosome technology.

MATERIALS AND METHODS

Preliminary Work

Collection of Plant material: The leaves of *Azadirachta indica* was collected in the month of January from Bhopal, region Madhya Pradesh, India.

Drying and Size Reduction of Plant Material: leaves of *Azadirachta indica* were dried under shade in laboratory. They were pulverized to make coarse powder. The coarse powder of leaves was passed through sieve No. 18 to maintain uniformity and stored in cool and dry place for study.

Screening of Powder (Physiochemical Analysis): Physiochemical screening of powdered leaves under the parameters Loss on Drying, Total Ash Value, Acid Insoluble Ash Value, Water Soluble Ash Value and Foaming Index was done by the standard reported methods.

Preparation of *Azadirachta indica* leaves extract

(a) Extraction of leaves of *Azadirachta indica:* Extraction of leaves of *Azadirachta indica* was done by Soxhlet extraction method.

(b) Soxhlet Extraction: Soxhlet apparatus was used for the solvent extraction and methanol was selected as a solvent for extraction while petroleum ether was used for defatting of waxy materials.

Phytochemical Screening: extract obtained after extraction were subjected for phytochemical screeningto determine the presence of following various phytochemical present in the extracts.

Quantitative studies of phytoconstituents

(A) Estimation of total phenol content: The total phenol content of the extract was determined by the modified folin-ciocalteu method. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10- 50µg/ml was prepared in methanol. 2 ml of extract and each standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l)of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

(B) Estimation of total alkaloids content: The plant extract (1mg) was dissolved in methanol, added 1ml of 2 N HCl and filtered. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1-, 2-, 3- and 4-ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (40, 60, 80, 100 and 120 µg/ml) were prepared in thesame manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/100mg ofextract.

Preparation of Phytsomes of *Azadirachta indica* extract

Accurately weighed quantity of phosphatidylcholine and cholesterol were dissolved in 10 ml of chloroform in round bottom flask (RBF) and sonicated for 10 min using bath sonicator. Organic solvent removal is done by Rotary evaporator (45-50°C). After complete removal of solvent thin layer of phospholipids mixture was formed. This film was hydrated with methanolic extract of neem leaves in rotary evaporator (37-40°C for 1 hour). After hydration, mixture of lipid and plant extract was sonicated for 20 minutes in presence of ice bath for heat dissipation. Then prepared phytosomes were filled in amber colored bottle and stored in freezer (2-8^{0}C) until used.

Characterization of Phytsomes of *Azadirachta indica* extract

A. Visualisation: The morphology of phytosomes was observed by digital microscopy, transmission electron microscope and scanning electron microscope.

Digital Microscopy: Phytosome formulation shaken in distilled water and viewed under digital microscope at 400X objective lens. All the batches prepared were analyzed for particle size by optical microscope.

SEM Analysis: Approximately 5 µL of the phytosomal suspension was transformed to a cover slip, which in turn was mounted on a specimen tab. The samples were allowed to dry at room temperature. Then the particle

size of the formulation was viewed and photographed using Scanning Electron Microscope (Sigma, Carl Zeiss).

B. FTIR: spectral data were taken to ascertain the structure and chemical stability of extract, PC and phytosome. Spectral scanning was done in the range between 4000 and 500 cm^{-1}

C. Drug Content & Encapsulation Efficiency: 20 mg of the microspheres from each batch were taken and digested in 100 ml of 0.1N HCl in a 100 ml volumetric flask and kept aside with intermittent shaking for 24 h. Then, the contents of the flask were filtered by using Whatman filter paper no.1. Then 1 ml of the filtrate was diluted with 50 ml of dimethyl sulfoxide (DMSO) in a volumetric flask and sonicated for 10 min so that leave out neem extract from phytosome. This was again filtered by using Whatman filter paper no.1; one ml from this was further diluted with methanol up to 10 ml and absorbance measured at 330 nm using methanol as blank. After recording the absorbance, the drug content and encapsulation efficiency were calculated. The readings were taken thrice and the average reading was taken for further calculation.

D. *In-Vitro* Drug Release Studies: The in vitro dissolution studies were carried using USP - 34 paddle type dissolution apparatus. 50 mg neem extract loaded phytosomes were placed in a dialysis bag and introduced into100 ml dissolution medium of buffer solution pH 7.4 maintained at 37± 0.5 ºC at a rotation speed of 50 RPM. 1 ml of aliquots was withdrawn at predetermined time intervals and an equivalent volume of fresh medium was replaced to maintain sink condition. The aliquots were diluted and analyzed spectrophotometrically at 330nm to determine the concentration of drug present. The readings were taken thrice and the average reading was taken for further calculation.

Accelerated stability studies

The above prepared samples were kept in sealed vials for 7 days at 40 ºC and 75% RH.

Preparation of Neem extract loaded phytosomal gel

Dissolve different concentrations of HPMC in ethanol and propylene glycol in water were mixed together using a magnetic stirrer, at 25 rpm. By keeping Neem extract loaded phytosome concentration constant in all the formulations. The Neem extract loaded phytosome was poured into the polymer solution. The solution was kept under stirring and then the pH was adjusted using 0.1M NaOH and the formulated gel was taken for further analysis.

Evaluation of prepared Gel

Physical examination: The formulation was manually examined to check any variations in the color, odor, and texture.

Determination of pH: pH of each formulation was determined by using pH meter (pH meter Toshconcl 54+) which was calibrated before with buffer solutions of pH 4, 7 and 9.

Determination of Viscosity: Viscosity of each formulation was determined using Brookfield viscometer with spindle at room temperature and at 5, 10, 20, 50 and 100 rpm.

Drug content: 0.2 gm of the gel formulation (equivalent to 10 mg of drug) was taken in 100 ml volumetric flask which contains 20 ml of phosphate buffer pH 7.4 and sonicated for 15 minutes. Volume was made upto 100 ml. 1ml of above solution was further dilute to 10 ml by using phosphate buffer of pH 7.4. The resultant solution was subjected to UV spectrophotometric analysis at 330 nm and the absorbance was noted down.

Spreadability: To determine spreadability of the gel formulations, two glass slides of known standard dimensions are selected. Formulation whose spreadability to be determined was place on one slide and then other slide was kept over its top such that the gel is sandwiched between the two slides. The slides were pressed upon each other so as to displace any air present, and the adhering gel was wiped off. The two slides were placed onto a stand such that only the lower slide is held firm by the one opposite fangs of the clamp clips and allows the upper slide to slip freely over it by the force of weight tied Tie the 20-gm weight to the upper slide carefully. The time taken by the upper slide to completely detach from the lower slide was noted. The spreadability was calculated by using the following formula.

$$s = m \cdot \frac{l}{t}$$

Value 's' is spreadibility, m is the weight tied to the upper slides, l is the length of glass slide, and t is the time taken.

***In-vitro* permeation study:** The diffusion of Acyclovir from gel formulations was studied through egg membrane and cellophane membrane using the Franz diffusion apparatus (figure 7.1). The donor cell was filled with 300 mg of gel formulation (equivalent to 15 mg of drug). The receptor compartment is filled by phosphate buffer having pH 7.4. Temperature of the receptor compartment was maintained at 37 ± 0.5ºC by using circulation of hot water through the jackets of Franz diffusion cell. The samples were removed at predetermined intervals at 0.5,1,2,4,6 hours and replaced immediately with equal volume of receptor solution to maintain sink conditions. The removed samples were analyzed at 330 nm on UV spectrophotometer.

Stability study

Stability study is performed for F-3 as the formulation shows greatest drug release and hence can be termed as 'best formulation' from within those that are developed. Stability study was carried out for 1 month; the formulation was kept in stability chamber at 40ºC and at 75% relative humidity and 4 ºC. After one month the formulation was checked for parameters like phase separation pH and drug content.

RESULT & DISCUSSION

Morphological Characterization of *Azadirachta indica* leaves: Leaves of *Azadirachta indica* were green in color, bitter in taste, Length – 1.5-3cm, Width -1-1.5cm in size, ovate in shape and Rough outer periphery.

Physiochemical analysis of *Azadirachta indica* leaves powder

Table No.1: Physiochemical analysis of powder of *Azadirachta indica* leaves

S. No.	Parameters	Observation (%)
1	Total ash value	9
2	Loss on drying	1.2
3	Acid insoluble ash value	2.9
4	Water soluble ash value	1.6
5	Foaming index	6 (ml)

Extract of *Azadirachta indica:* Extractive values of Pet. ether extracts of *Azadirachta indica were* % Yield (2.91% w/w) , Dark green Color, greasy in Consistency and methanol extracts of *Azadirachta indica were* % Yield (6.34% w/w), Dark green Color, semi solidConsistency.

Table No. 2: Phytochemical screening of methanolic extract of *Azadirachta indica* leaves

S. No.	Chemical Tests	Ethanolic extract
1	**Carbohydrates** i) Molisch's Test ii) Fehling's Test iii) Benedict's test	 (+) (-) (+)
2	**Tannins** i) with 5%ferric chloride solution ii)with 10% aqueous Potassium dichromate solution iii) with 10% lead acetate solution	 (-) (-) (-)
3	**Alkaloids** i)Dragendorff's Test ii) Mayer's Test iii) Hager's Test	 (-) (+) (+)
4	**Glycosides** i) Borntrager's Test ii) Legal Test iii) Baljet Test	 (+) (–) (-)
5	**Flavonoids** i) Shinoda's Test ii) Alkaline reagent test iii) Lead test	 (+) (+) (+)

6	**Steroids and Sterols** i) Libermann-Burchard Test ii) Salkowski Test	 (-) (-)
7	**Proteins and Amino Acids** i) Biuret Test ii) Ninhydrin Test iv) Millon's Test	 (–) (-) (+)

(+) = Present, (–) = Absence

Estimation of total phenol and alkaloid content in extract of *Azadirachta indica* leaves

Total Phenolic content estimation (TPC): Total phenolic compounds (TPC) was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: y = 0.019x + 0.020, R^2= 0.998, where X is the gallic acid equivalent (GAE) and Y is the absorbance.

Table 3: Preparation of Calibration curve of Gallic acid

S. No.	Concentration (µg/ml)	Mean Absorbance
1	0	0
2	10	0.226
3	20	0.412
4	30	0.614
5	40	0.803
6	50	0.966

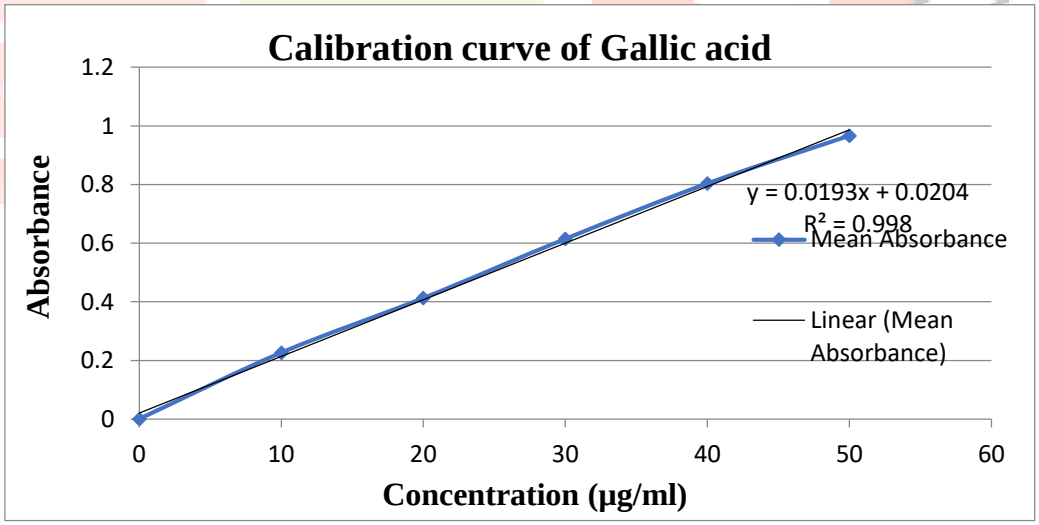

Figure 1: Graph of Calibration Curve of Gallic acid

Total alkaloid content estimation (TAC): Total alkaloid content was calculated as atropine equivalent mg/100mg using the equation based on the calibration curve: y = 0.008x + 0.010, R^2= 0.999, where X is the Atropine equivalent (AE) and Y is the absorbance.

Table 4: Preparation of calibration curve of Atropine

S. No.	Concentration (µg/ml)	Mean Absorbance
1	**0**	**0**
2	40	0.352
3	60	0.514
4	80	0.679
5	100	0.845
6	120	0.997

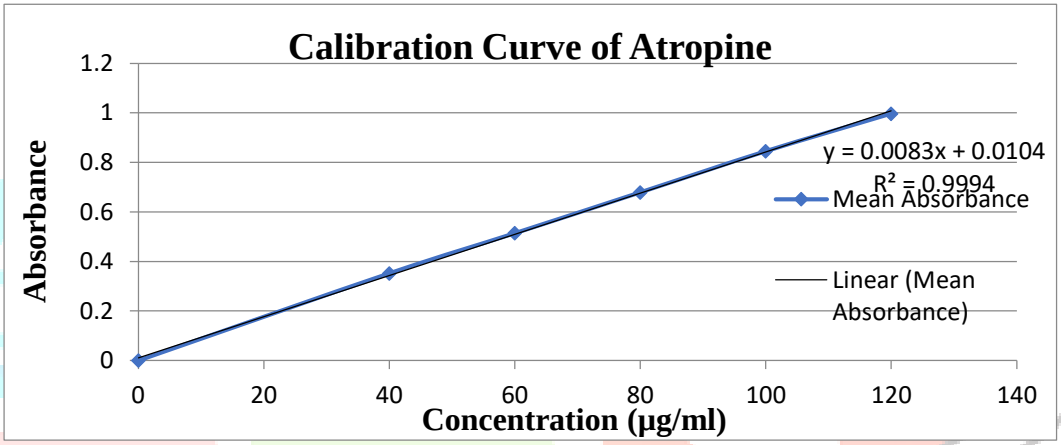

Figure 7.3: Graph of Calibration Curve of Atropine

Table 5: Estimation of total phenolic and alkaloid content *Azadirachta indica* extract

S. No.	Extract	Total phenol content (mg/100mg of dried extract)	Total alkaloid content (mg/ 100 mg of dried extract)
1.	Methanol	0.756	0.632

Formulation of Phytosome of neem leaves extract:

Five different type formulations formed using different concentration of drug and polymer.

Table No 6: Formulations formed using different concentration of drug and polymer

Formulation code	Neem leaves extract (mg)	Phosphatidylcholine (PC) (mg)	Cholesterol (CL)(mg)	Phytosome PC:CL
F-1	200	100	25	4:1
F-2	200	100	50	2:1
F-3	200	100	75	4:3
F-4	200	100	100	1:1
F-5	200	100	125	1:1.25

Characterization of Neem Extract Loaded Phytosome

Table No.7: Characterization of Neem Extract Phytosome

Batch Code	Yield (%)	Drug Content (%)	Mean Particle Size(nm)	Encapsulation Efficiency (%)
F1	89.12 ± 0.05	90.21 ± 0.21	732 ± 13	78.67 ± 1.52
F2	88.32 ± 0.08	92.35 ± 0.76	694 ± 29	84.27 ± 0.81
F3	98.91 ± 0.03	97.52 ± 1.90	637 ± 17	95.34 ± 0.64
F4	94.45 ± 1.06	91.41 ± 1.63	713 ± 44	92.52 ± 1.30
F5	93.48 ± 1.95	90.44 ± 1.02	694 ± 21	92.19 ± 1.68

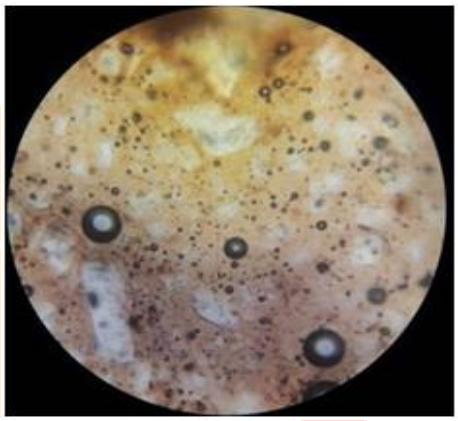

Figure 7.5: Microscopic observation of optimized batch F-3

Drug: Excipient Compatibility by FT-IR Study

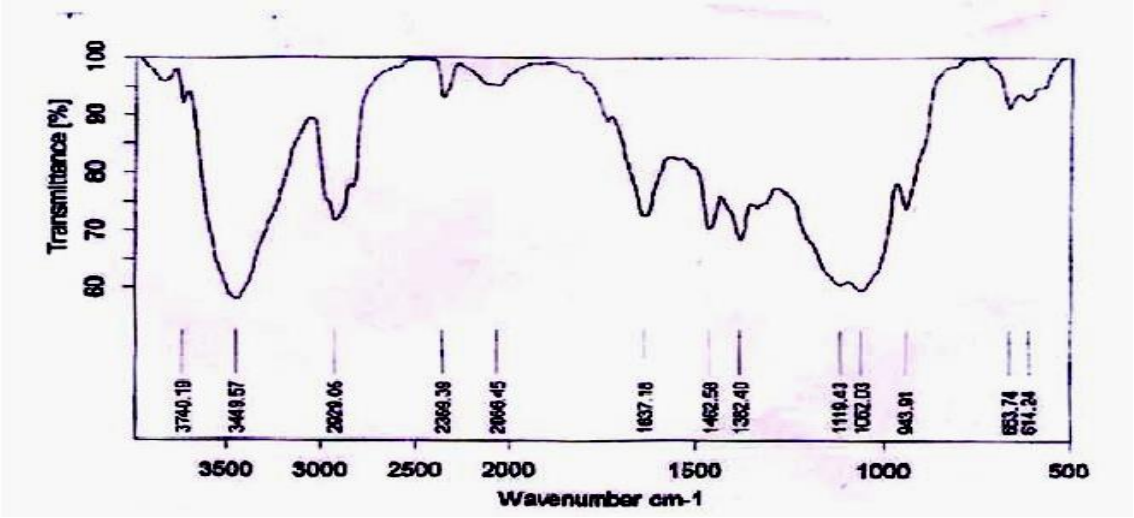

Figure 5: FT-IR of Neem Leaves Extract

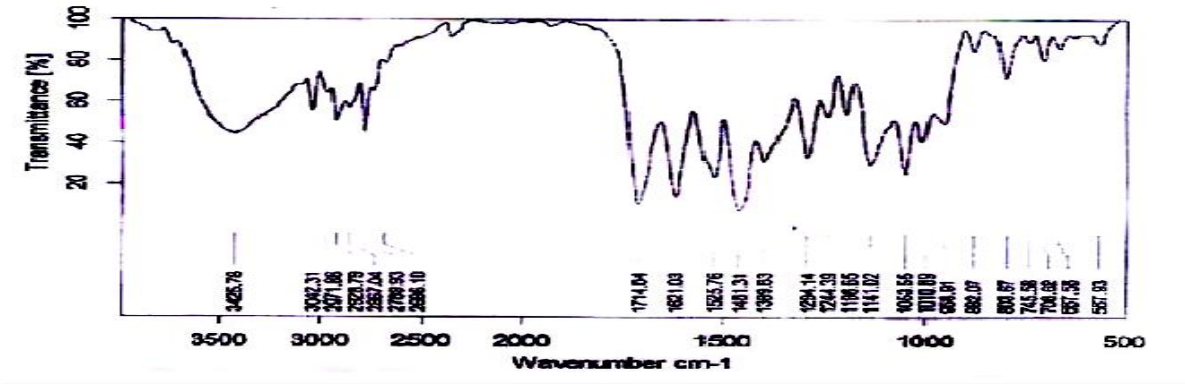

Figure 6: FT-IR of Neem Leaves Extract and Additives

Table No. 14: FT-IR Peaks of Neem Leaves Extract

Standarded Peaks (Cm^{-1})	Observed Peaks (Cm^{-1})	Peak Assignments
3050-3500	3449	O-H str
3000-2840	2829	CH_3str
1650-1600	1637	C=C str
1750-1700	1720	C=O str (Carboxylic acid)
1392-1366	1362	N-H str

Str. = Stretching

Formulation of topical gel of Neem extract loaded Phytosome

Table No. 17: Formulation of Topical Gel of Neem Extract Loaded Phytosome

Formulation	F-1	F-2	F-3	F-4	F-5
Phytosome(g)	0.4	0.4	0.4	0.4	0.4
HPMC(g)	0.5	0.5	0.5	0.5	0.5
Ethanol (ml)	5	5	5	5	5
Propylene glycol (g)	1	1	1	1	1
Distilled water (g)	3.1	3.1	3.1	3.1	3.1

Evaluation of Neem Extract Loaded Phytosome gel

Table No. 18: Physical Evaluation of Neem Extract Loaded Phytosome gel

Formulation code	Clarity	Odor	Phase Separation	Wash ability	Homogeneity	Grittiness
F-1	Clear	No	No	Washable	Yes	No
F-2	Clear	No	No	Washable	Yes	No
F-3	Clear	No	No	Washable	Yes	No
F-4	Clear	No	No	Washable	Yes	No
F-5	Clear	No	No	Washable	Yes	No

Table No. 19: Evaluation of Neem Extract Loaded Phytosome gel

Formulation code	pH	Spread-ability(cm)	% Drug Content	Viscosity(cp)	% Permeation
F-1	6.9	5.6 ± 0.3	99.9 ± 1.2	110 ± 1.8	83.2%
F-2	6.8	6.8 ± 0.2	99.6 ± 2.1	113 ± 2.0	86.3%
F-3	7.1	7.1 ± 0.6	98.9 ± 6.1	115 ± 1.2	92.7%
F-4	7.3	7.6 ± 0.2	99.8 ± 5.7	100 ± 0.8	91.0%
F-5	7.1	7.9 ± 0.6	101 ± 0.2	98 ± 2.6	90.1%

In-Vitro Drug Release Profile of Neem Extract Loaded Phytosome gel

Table No 21: *In-Vitro* Drug Release Profile of Phytosome gels

Time (T) (Hr.)	%C. R. F-1	%C. R. F-2	%C. R. F-3	%C. R. F-4	%C. R. F-5
0	0	0	0	0	0
1	17.249	19.62	21.6	22.68	24.3
2	29.835	31.68	33.12	30.726	33.255
4	32.34	39.68	44.64	41.876	37.399
6	44.566	48.7	49.692	48.227	42.645
8	50.931	59.22	60.405	49.932	50.979
10	60.57	65.62	70.276	55.785	55.758
12	78.541	82.18	73.72	61.489	60.922
16	81.49	84.6	81.681	67.403	64.853
18	84.273	88.56	87.011	72.808	69.164
20	88.329	93.18	93.092	76.621	74.577
24	92.765	95.56	97.913	81.533	80.018

Figure 9: Zero order plots of all five Formulations

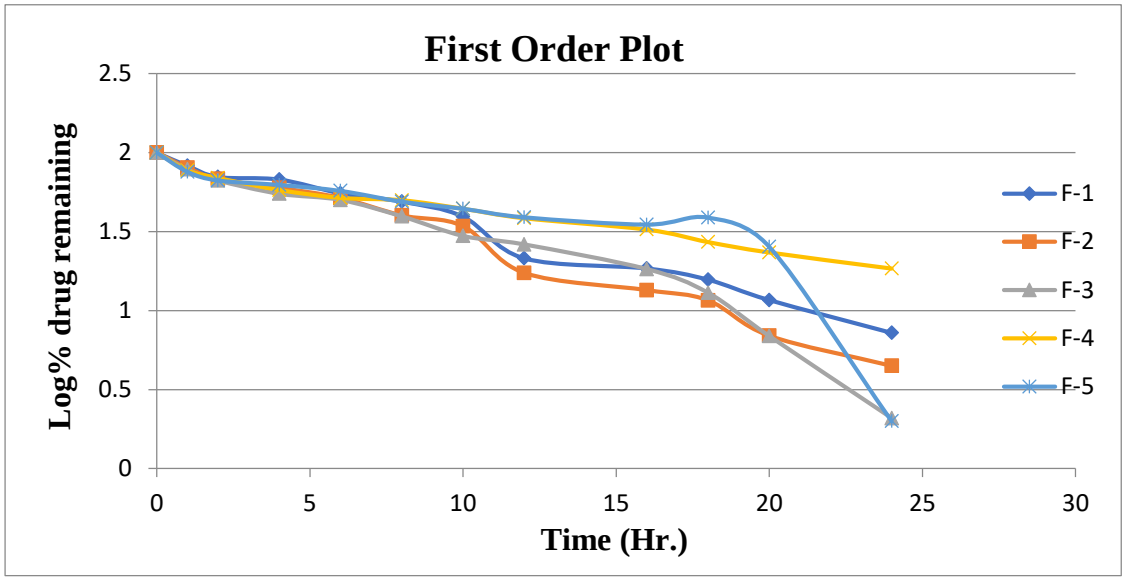

Figure: 10: First order plots of all five Formulations

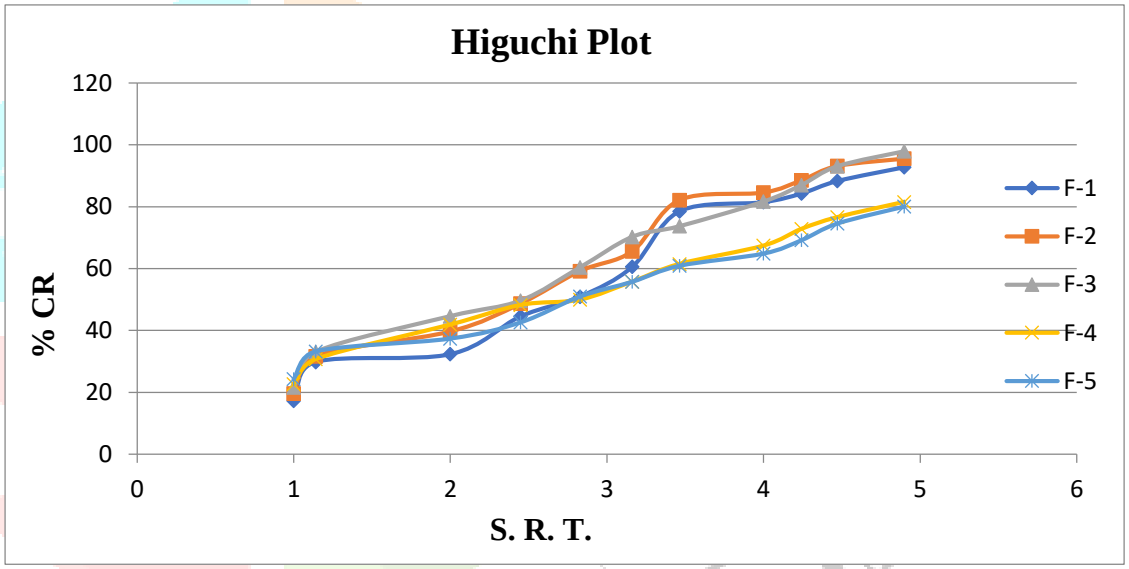

Figure 11: Higuchi plots of all five Formulations

Figure : R^2 Values of different of formulations in different type of plots

Formulation code	Zero Order	First Order	Higuchi
F-1	0.812	0.982	0.973
F-2	0.893	0.984	0.981
F-3	0.898	0.935	0.990
F-4	0.871	0.977	0.981
F-5	0.872	0.650	0.974

Stability study of Neem extract loaded Phytosome gel

Table No. 20: Stability of Neem extract loaded Phytosome gel

Formulation code	Phase separation		pH		Drug content (%)	
	4ºC	40 ºC	4ºC	40 ºC	4ºC	40 ºC
F-1	No	No	7.1	7.3	100 ± 1.6	98 ± 1.6
F-2	No	No	7.2	7.1	100 ± 1.1	99 ± 1.3
F-3	No	No	7.0	7.1	99 ± 1.3	99 ± 1.1
F-4	No	No	6.5	7.1	98 ± 0.9	96 ± 0.6
F-5	Yes	Yes	6.9	6.2	99 ± 0.3	94 ± 1.1

Discussion

Phytosomes of neem leave extract has been successfully formulated in this procedure firstly *Azadirachta indica* leaves were morphological Characterized. Neem leave powder was subjected for various physiochemical analysis like total ash value (9), Loss on Drying (1.2), Acid Insoluble Ash Value (2.7), Water Soluble Ash Value (1.6) and Foaming Index (6 ml) and then extracted out methanolic fraction of neem leaves, yield of the extract which was 6.34 %. Phytochemical screening found that carbohydrate, alkaloids and flavonoids were present.

Estimation of total phenol and alkaloid content in extract of *Azadirachta indica* leaves. Total phenolic compounds (TPC) was 0.756mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: $y = 0.019x + 0.020$, $R^2 = 0.998$, where X is the gallic acid equivalent (GAE) and Y is the absorbance. Total alkaloid content was atropine equivalent 0.632mg/100mg using the equation based on the calibration curve: $y = 0.008x + 0.010$, $R^2 = 0.999$, where X is the Atropine equivalent (AE) and Y is the absorbance.

Five different types of phytosome formulations were formed using different ratio of Phosphatidylcholine and Cholesterol (4:1), (2:1), (4:3), (1:1) and (1:1.25). These formulations were characterized under various parameters like yield, drug content, particle size and Encapsulation Efficiency. For all formulation yield was 88.32 % to 98.91%, drug content was 90.21% to 97.52 %, Mean Particle Size(nm) about 700 nm and Encapsulation Efficiency was 78.67% to 95.34%. Drug: Excipient Compatibility confirmed by FT-IR studies. All five phytosome formulations were incorporated with gel and formed clear, odorless, washable, homogeneous, stable and free from grittiness gel was evaluated under the various parameter, pH of all formulations was observed between 6.8 to 7.3 and Spreadability between 5.6 to 7.9 cm. % drug content between 98.9 % to 101%, and viscosity between 98 to 115 centi poice (cp) and % permeation between 83.2 % to 92.7 %. But on the basis of drug release kinetics **F-3** formulation was very good because its % drug release was **97.913%** followed **Higuchi** Kinetic Model. With the 95.56% drug release **F-2** formulation was good both follow First order

Kinetics. Other three formulation drug releases were F-1 (92.765) F-4 (81.467) and F-5 (80.018). Four gel formulations were F-1 to F-4 are stable but F-5was not stable at 4ºC and 40 ºC and phase separation occurs.

CONCLUSION

Neem leaves collected and extracted under soxhelate apparatus then physicochemical and phytochemical analysis was performd and characterize the drug .preformulation study was performed under the points as solubility studies, partition coefficient and drug excipient compatibility by FTIR and DSC analysis method.then after five different formulation of phytosomes was formulated and phytosomes were evaluated on various parameter like visuality, drug content, encapsulation efficiency and % drug release. Then all five types phytosomes were incorporated into gel and evaluated under gel parameter like clarity, griteeness, spredability, drug content, and diffusion study. Based on release kinetics formulation F-3 was very good formulation and F-2 was also good formulation.

CONFLICTS OF INTERESTS

There are no conflicts of interests.

REFERENCES

1. Vaishali Jadhav, Vijaya Bhogale “Novel drug delivery system in herbal” International Journal of Pharma Wave. Vol. 1, Issue 2, 2015.
2. Park K. Controlled drug delivery systems: Past forward and future back. J Control Release 2014;190:3-8.
3. Sharma S, Sikarwar M. Phytosome: A Review. Planta Indica .2005; 1:1-3.
4. Nilesh Jain, Brahma P Gupta, Navneet Thakur, Ruchi Jain, Jitendra Banweer, Deepak Kumar Jain, Surendra Jain “Phytosome: A Novel Drug Delivery System for Herbal Medicine” International Journal of Pharmaceutical Sciences and Drug Research 2010; 2(4): 224-228.
5. Yanyu X, Yunmei S, Zhipeng C, Quineng P. The Q preparation of Silybin phospholipidcomplex and the 1 study on its pharmacokinetics in rats. Int J Pharm 2006; 307:77-82.
6. Niyaz BB, Kalyani P, Divakar G: Formulation and evaluation of gel containing fluconazole-antifungal agent. International Journal of Drug Development and Research. 2011; 3(4): 109-128
7. Goyal S, Sharma P, Ramchandani U, Shrivastava SK and Dubey PK: Novel antiinflammatory topical gels. International Journal of Pharmaceutical and Biological Archives. 2011; 2(4): 1087-1094.
8. Nguyen Duc Hanh, Le Van Thinh, Do Quang Duong. Development and Evaluation of Neem Gel Formulation using Gum Karaya as Gelling Agent. Research J. Pharm. and Tech. 2020; 13(4): 1859-1864.
9. Raju D, Jose J. Development and evaluation of novel topical gel of neem extract for the treatment of bacterial infections. J Cosmet Dermatol. 2019;18(6):1776-1783.

10. Narendra Ahirwal and Dr. S. K. Nayak. Formulation and evaluation of phytosomal gel of neem. World Journal of Pharmaceutical Research. 2021; 10(6):1177-1198.